

We claim:

1. A genetically engineered methylotrophic yeast strain which produces glycoproteins comprising a mammalian-like N-glycan structure, wherein said
5 mammalian-like N-glycan contains five or fewer mannose residues and at least one N-acetylglucosamine residue (GlcNAc) which is linked to a mannose residue and to a terminal galactose residue.
2. The strain of claim 1, wherein said mammalian-like N-glycan comprises
10 GalGlcNAcMan₅GlcNAc₂.
3. The strain of claim 1, wherein said strain is a strain of the genera *Candida*, *Hansenula*, *Torulopsis*, or *Pichia*.
- 15 4. The strain of claim 3, wherein said strain is a *Pichia pastoris* strain.
5. The strain of claim 2, wherein said strain expresses (1) an α -1,2-mannosidase or a functional part thereof, (2) an N-acetylglucosaminyltransferase I (or GnTI) or a functional part thereof, and (3) a β -1,4-galactosyltransferase (GalT) or a
20 functional part thereof.
6. The strain of claim 2, wherein the genomic OCH1 gene is inactivated.
7. The strain of claim 5, wherein said α -1,2-mannosidase or said functional part
25 thereof is of an origin of a mammalian species or a fungal species.
8. The strain of claim 7, wherein said mammalian species is selected from murine, rabbit or human.

9. The strain of claim 7, wherein said fungal species is selected from *Aspergillus* or *Trichoderma reesei*.
- 5 10. The strain of claim 5, wherein said α -1,2-mannosidase or said functional part thereof is targeted to the ER or the Golgi of said strain.
11. The strain of claim 10, wherein said α -1,2-mannosidase or said functional part thereof is engineered to contain an ER-retention signal.
- 10 12. The strain of claim 11, wherein said ER-retention signal comprises HDEL (SEQ ID NO: 1).
13. The strain of claim 5, wherein said GnTI or said functional part thereof is of an origin of a species selected from the group consisting of rabbit, rat, human, plant, insect, nematode and protozoa.
- 15 14. The strain of claim 13, wherein said GnTI or said functional part thereof is of a human origin.
- 20 15. The strain of claim 5, wherein said GnTI or said functional part thereof is targeted to the Golgi apparatus of said strain.
16. The strain of claim 15, wherein said GnTI or said functional part thereof is engineered to contain a Golgi-retention signal.
- 25 17. The strain of claim 16, wherein said Golgi-retention signal comprises SEQ ID NO: 11.

18. The strain of claim 5, wherein said GalT or said functional part thereof is of an origin of a species selected from the group consisting of rabbit, rat, human, plant, insect and nematode.
- 5 19. The strain of claim 18, wherein said GalT or said functional part thereof is of a human origin.
20. The strain of claim 5, wherein said GalT or said functional part thereof is targeted to the Golgi apparatus of said strain.
- 10 21. The strain of claim 20, wherein said GalT or said functional part thereof is engineered to contain a Golgi-retention signal.
22. The strain of claim 21, wherein said Golgi-retention signal comprises SEQ ID NO: 11.
- 15 23. The strain of claim 5, wherein said α -1,2-mannosidase or said functional part is expressed from a promoter selected from the group consisting of the AOXI promoter, the AOXII promoter, the GAP promoter, and the FLD promoter of *Pichia pastoris*.
- 20 24. The strain of claim 5, wherein said GnTI or said functional part is expressed from a promoter selected from the group consisting of the AOXI promoter, the AOXII promoter, the GAP promoter, and the FLD promoter of *Pichia pastoris*.
- 25 25. The strain of claim 5, wherein said GalT or said functional part is expressed from a promoter selected from the group consisting of the AOXI promoter, the AOXII promoter, the GAP promoter, and the FLD promoter of *Pichia pastoris*.

26. The strain of claim 5, wherein α -1,2-mannosidase or said functional part is expressed from the AOX1 promoter of *Pichia pastoris*, and said GnTI or said functional part is expressed from the GAP promoter of *Pichia pastoris*.
- 5 27. The strain of claim 1, wherein said mammalian-like glycan is a human-like glycan.
28. A method of recombinantly producing a glycoprotein having a mammalian-like N-glycan structure, comprising transforming a strain according to any one of
10 claims 1-2 or 5-6 with a nucleotide sequence coding for said glycoprotein, and expressing said glycoprotein in said strain.
29. The method of claim 28, wherein said glycoprotein is a protein of a bacterial, fungal, viral or mammalian origin.
- 15 30. A glycoprotein produced by the method of claim 28.
31. A method of recombinantly producing a glycoprotein having a mammalian-like N-glycan structure in a methylotrophic yeast strain, comprising transforming said
20 strain with a nucleotide sequence coding for said glycoprotein; modifying said strain such that the modified strain expresses (1) an α -1,2-mannosidase or a functional part thereof, (2) an N-acetylglucosaminyltransferase I (or GnTI) or a functional part thereof, and (3) a β -1,4-galactosyltransferase (GalT) or a functional part thereof, and wherein the genomic OCH1 gene in the modified
25 strain is inactivated; and producing said glycoprotein in the modified strain.
32. The method of claim 31, wherein said glycoprotein is selected from a protein of a bacterial, fungal, viral or mammalian origin.

33. A glycoprotein produced by the method of claim 31.
34. A vector comprising a nucleotide sequence coding a GalT, operably linked to a promoter sequence and a 3' termination sequence, wherein said promoter sequence and said 3' termination sequence are functional in a methylotrophic yeast strain to achieve expression of said GalT in said strain.
35. A kit comprising (1) a vector which comprises a nucleotide sequence coding for an α -1,2-mannosidase or a functional part thereof, (2) a vector which comprises a nucleotide sequence coding for a GnTI or a functional part thereof, and (3) a vector which comprises a nucleotide sequence coding for a GalT or a functional part thereof, wherein each of the vectors is capable of directing the expression of the encoded protein in a methylotrophic yeast strain.
36. The kit of claim 35, further comprising a vector capable of disrupting the genomic OCH1 gene of said methylotrophic yeast strain.
37. The kit of claim 35, further comprising a vector coding for a glycoprotein heterologous to said methylotrophic yeast strain.
38. The kit of claim 35, further comprising said methylotrophic yeast strain.